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DECREASED ATTRACTION OF *Anastrepha ludens* TO COMBINATIONS OF TWO TYPES OF SYNTHETIC LURES IN A CITRUS ORCHARD

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Abstract—Combinations of the previously developed attractants CEHO from host fruit aroma and AMPu from volatile metabolites of amino acids were evaluated for attractiveness to gamma-irradiated Mexican fruit flies, *Anastrepha ludens*, in a citrus orchard. In one experiment, McPhail traps with polyvinyl chloride (PVC) lures loaded with CEHO (10 : 1 : 1 : 50 mixture of 1,8-cineole, ethyl hexanoate, hexanol, and ethyl octanoate) were more attractive than blank traps. McPhail traps with AMPu (10 : 10 : 1 mixture of ammonium bicarbonate, methylamine HCl, and putrescine) were more attractive than blank and CEHO traps. Traps containing both AMPu and CEHO lures were less attractive than traps containing AMPu alone. In another experiment, sticky yellow panels and spheres were used to compare attractiveness of the same two attractants in different formulations. Sticky traps baited with membrane formulations of CEHO (10 : 1 : 1 : 10 mixture of the chemicals listed above) were not significantly attractive. Sticky traps with polypropylene tubes containing an agar formulation of AMPu (6 : 10 : 1 mixture of ammonium carbonate, methylamine HCl, and putrescine) were more attractive than blank and CEHO baited traps. As in the first experiment, traps with both AMPu and CEHO lures were less attractive than traps with AMPu alone. Results indicate that attractive host-odor volatiles and attractive amino-acid metabolites interact negatively with regard to attractiveness to the Mexican fruit fly.

Key Words—Attractants, Mexican fruit fly, Diptera, Tephritidae, *Anastrepha ludens*, host odors, ammonia, amines.

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INTRODUCTION

In the past five years, development and laboratory bioassay of two different types of attractants were reported for the Mexican fruit fly, *Anastrepha ludens* Loew. The first was a four-component attractant (CEHO) developed from yellow chapote fruit (*Sargentia greggii* S. Wats.), a natural host of the fly (Robacker et al., 1992). The second was a three-component attractant (AMPu) containing metabolites from biological degradation of amino acids (Robacker and Warfield, 1993). Both of these attractants were more attractive than *Torula* yeast, the most commonly used bait for Mexican fruit flies in flight chamber tests in a greenhouse. In tests in a citrus orchard both were significantly more attractive than blank traps, but AMPu was not more attractive than *Torula* yeast (Robacker, 1995), and CEHO was less attractive than *Torula* yeast (Robacker and Heath, 1996). The purpose of this work was to determine if combinations of CEHO and AMPu would be more attractive than either alone.

METHODS AND MATERIALS

Insects. Flies were from a culture that had been maintained on laboratory diet for about 100–120 generations with no wild-fly introductions. Flies were irradiated with 70–92 Gy (^{137}Cs source) one to two days before adult eclosion, to comply with quarantine laws for releasing *A. ludens*. Mixed-sex groups of 180–200 flies were kept in 473 ml cardboard cartons with screen tops until used in tests. Laboratory conditions for holding flies were 22 ± 2°C, 50 ± 20% relative humidity, and photophase from 06:30 to 19:30 hr provided by fluorescent lights. Flies were fed sucrose and water until the time of release. Flies were released into the orchard when 2–10 days old during the late afternoon of the day before a test. Approximately 2000 flies were distributed equally in the test area of the orchard.

Test Chemicals and Formulations. Two types of CEHO lures were used. One was a polyvinyl chloride (PVC) formulation prepared by Scentry, Inc. (now Ecogen, Inc., Langhorne, Pennsylvania). These lures contained 1,8-cineole, ethyl hexanoate, and ethyl octanoate obtained from Aldrich Chemical Co., Inc., (Milwaukee, Wisconsin), and hexanol obtained from Sigma Chemical Company (St. Louis, Missouri). All four chemicals were >99% pure. Each lure contained 120 mg of a 10:1:1:50 mixture of 1,8-cineole, ethyl hexanoate, hexanol, and ethyl octanoate, respectively. These PVC lures were more attractive than *Torula* yeast in flight-chamber experiments (Robacker et al., 1992).

The second CEHO lure was a membrane-based formulation like that described previously for synthetic pheromone of the papaya fruit fly (*Toxotrypana curvicauda* Gerstaecker) (Heath et al., 1996). Briefly, the lure was a

3- × 5-cm envelope made from impermeable polyethylene containing a high-density polyethylene membrane (Consep Inc., Bend, Oregon). Chemicals diffusing through the membrane were released from the lure through a 1.17-cm-diam. hole on one side of the envelope. Two loadings of the chemicals were used. The low loading was 25 μl of a 10 : 1 : 1 : 10 mixture of the chemicals; and the high loading was 100 μl of the same mixture. Chemicals were obtained from Aldrich and were >99% pure. McPhail traps containing these membrane formulations were significantly more attractive than water blanks and significantly less attractive than *Torula* yeast in citrus orchard experiments (Robacker and Heath, 1996).

AMPu also was tested in two formulations. One formulation was an aqueous solution of ammonium bicarbonate, methylamine HCl, and putrescine. The chemicals were obtained from Sigma and were at least 98% pure. Concentrations of the three chemicals in the formulation were 400 : 400 : 40 μg/ml, respectively, at a pH of 8.8. These concentrations were the most attractive in previous experiments (Robacker, 1995).

The second AMPu formulation was an agar gel for use in dry traps. Ammonium carbonate was substituted for ammonium bicarbonate because of its greater solubility in water, with the molar ratios of ammonia, methylamine, and putrescine the same as in the aqueous formulation. Ammonium carbonate was obtained from Aldrich and was at least 98% pure. The AMPu-agar formulation was prepared by mixing hot agar (Bacto Agar, Difco Laboratories, Detroit, Michigan) solution with aqueous AMPu in 1.9 ml polypropylene microcentrifuge tubes (A. Daigger & Company, Inc., Wheeling, Illinois). Final agar concentration was 1%. AMPu component concentrations in agar tubes were 60 mg/ml ammonium carbonate, 100 mg/ml methylamine HCl, and 10 mg/ml putrescine. Final pH of the AMPu tubes was 8.5–8.8. Sticky traps baited with AMPu-agar tubes prepared in this way were equal in attractiveness to *Torula* yeast in McPhail traps in earlier research (Robacker, 1995).

Experiment 1. Attractiveness of AMPu–CEHO Combinations in McPhail Traps. Two AMPu–CEHO combinations were evaluated against AMPu alone, CEHO alone, and a water blank in McPhail traps. PVC formulations of CEHO and aqueous formulations of AMPu were used in this experiment. PVC CEHO lures were used when 6–18 days old. Previous laboratory research indicated lures were most attractive between the ages of 10–15 days (Robacker et al., 1992) and preliminary field work indicated lures were actually repellent to flies before 6 days.

Traps with AMPu–CEHO combinations or AMPu alone contained 200 ml of the aqueous AMPu formulation in the trap reservoir. One AMPu–CEHO trap and the trap with CEHO alone contained a PVC CEHO lure suspended inside the trap 1–2 cm above the entrance hole on the underside of the trap. The trap with CEHO alone also contained 200 ml of water. The second AMPu–CEHO combination used a PVC CEHO lure fastened to the top on the outside of the trap. CEHO lures used in the three CEHO treatments were always of the same age within each replication of the experiment. All traps contained amber coloring in the water from a combination of red, yellow, and green food colors (McCormick & Co., Inc., Baltimore, Maryland) and 0.01% Triton (Rohm and Haas Co., Philadelphia, Pennsylvania) as a wetting agent.

The experiment was conducted in a mixed citrus orchard located near the laboratory in Weslaco, Texas. The orchard contained several varieties of orange, lemon, grapefruit, and tangerine trees of varying ages. One row of Ruby Red grapefruit (*Citrus paradisi*) and one row of Dancy tangerine (*C. reticulata*) were chosen for tests since they contained relatively large (2–3 m height) fruit-bearing trees. Two linear blocks of five consecutive trees each were used in each row, for a total of four blocks in the orchard. One each of the two AMPu–CEHO combinations, an AMPu trap, a CEHO trap, and a water blank trap were tested in each block. Each test lasted one day. Traps were placed in the orchard during the morning and removed for fly counts and cleaning on the following morning. Traps were hung one to a tree, north of center, at 1–2 m height.

Two series of one-day tests using two different sets of PV CEHO lures were conducted. In each series, one-day tests were conducted every two to four days. CEHO lures were suspended in a fume hood in the laboratory at 22 ± 2°C on nontest days. Four one-day tests were conducted in one series and five in the second for a total of 36 replications of each treatment (9 days × 4 blocks per day). Positions of treatments within each block were randomized for the first one-day test of each series. Positions of treatments in consecutive one-day tests were not randomized but were moved sequentially within each block. One series of one-day tests was conducted from August 9 to 20 and the second from October 1 to 13, 1993.

Experiment 2. Attractiveness of AMPu–CEHO Combinations on Sticky Traps. Four AMPu–CEHO–trap-type combinations were evaluated against various AMPu alone, CEHO alone, and blank trap controls. Membrane formulations of CEHO and agar formulations of AMPu were used in this experiment. CEHO lures were used between the ages of 0 and 30 days because previous research indicated attractiveness of these lures did not change as they aged up to one month (Robacker and Heath, 1996). AMPu lures were used only during the first day after removal from the refrigerator. The lures are highly attractive during this time (Robacker, 1995). Two types of sticky traps were used. One was a yellow ball trap (13 cm diam.) and the second was a yellow panel trap (13 × 18 cm). Traps were coated with Tangle-Trap (Tanglefoot Company, Grand Rapids, Michigan). Both traps had been evaluated previously with and without lures (Robacker, 1992, 1995). CEHO and AMPu lures were attached to the tops of the traps, on opposite sides when both lures were used on the same trap. Lure–trap combinations were: AMPu–CEHO (low loading)–ball;

AMPu–CEHO (low)–panel; AMPu–CEHO (high loading)–ball; AMPu–CEHO (high)–panel; AMPu–ball; AMPu–panel; CEHO (low)–ball; CEHO (low)–panel; CEHO (high)–ball; CEHO (high)–panel; unbaited ball; and unbaited panel.

The experiment was conducted in the same orchard as experiment 1 except that the four blocks were enlarged to contain eight trees each instead of five. One block in each of the two rows was used to test the four lure–trap combinations containing the low loading of CEHO, plus the two AMPu alone combinations, and the two blank traps. The other two blocks were used to test the four combinations containing the high loading of CEHO, plus the AMPu alone combinations and the blank traps. Experimental procedures were similar to experiment 1. Ten one-day tests were conducted from May 26 to July 27, 1994. Because two of the four blocks were used to test the low loading of CEHO and two were used to test the high loading, in essence two experiments were conducted at the same time. In each, 20 replications of each treatment were conducted.

Statistical Analyses. The numbers of males and females captured by individuals traps were transformed to log $(X + 1)$ to stabilize variance. Two-way analysis of variance (ANOVA) was conducted for both males and females for each experiment (Abacus Concepts, 1989) to test effects of test day, lure–trap type, and interactions of day with lure–trap type. For experiment 2, separate ANOVAs were conducted for blocks containing the low and high loadings of CEHO. Single degree-of-freedom contrasts were conducted to test effects of trap type (ball versus panel), lure type (AMPu versus water or blank, CEHO versus water or blank), and interactions of AMPu with CEHO.

Experiment 2 was reanalyzed after combining data from balls and panels and from blocks containing low and high loadings of CEHO. Replications over space (four blocks) were treated equivalently to replications over time (one-day tests). Two-way ANOVAs were conducted for both males and females to test lure effects and to remove effect of test day from the residual. The test day $\times$ lure interaction was left out of the models. Experiment 1 was reanalyzed similarly. Fisher's protected least significant difference (LSD) method was used to compare lure means. Paired t tests were conducted to test attractiveness of CEHO lures relative to water blanks within the same block and one-day test.

RESULTS

Experiment 1. Attractiveness of AMPu–CEHO Combinations in McPhail Traps. Test day $\times$ lure interactions were not significant according to ANOVAs for both males and females. ANOVAs (without test day $\times$ lure interaction terms) showed that McPhail traps containing aqueous AMPu were significantly more attractive than traps containing water or CEHO alone to both male and female

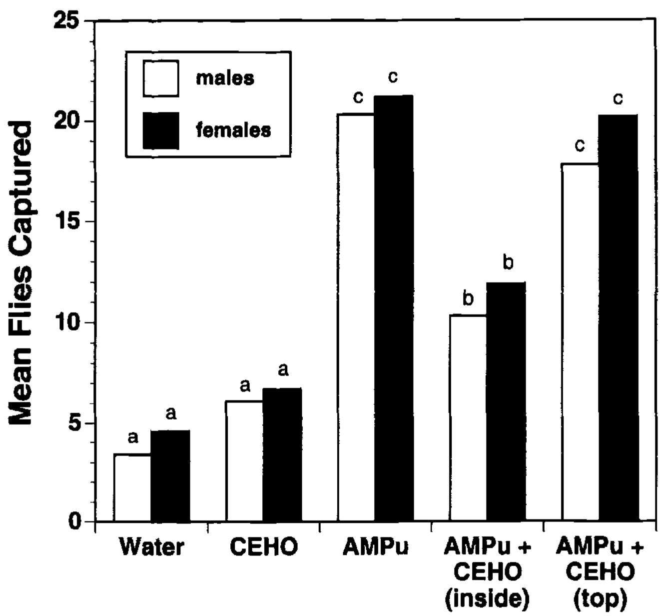

FIG. 1. Captures of *A. ludens* in McPhail traps baited with combinations of aqueous AMPu and PVC CEHO lures inside or on top of traps, compared with traps containing only water, a CEHO lure, or AMPu (experiment 1). Within each sex, bars with the same letter are not significantly different from each other by Fisher's protected LSD ($P < 0.05$; $N = 36$ for each trap/lure configuration).

flies (Figure 1) ($F = 46.9, 41.1$; $df = 4{,}167$; $P < 0.0001$ for males and females, respectively). Traps baited with CEHO alone were not significantly attractive by the LSD test. However, CEHO traps were more attractive than traps containing only water by a paired t test comparing traps within the same block, summed over males and females ($P < 0.05$, $t = 2.3$, $df = 34$). Traps baited with AMPu-CEHO combinations in which the CEHO PVC lure was located inside the trap were significantly less attractive than traps baited with AMPu alone to both males and females. Combinations with the CEHO lure on top of the trap were not significantly different from AMPu alone.

Experiment 2. Attractiveness of AMPu-CEHO Combination on Sticky Traps. Test day × lure interactions were not significant in any of the four two-way ANOVAs (highest $F = 1.1$; $df = 63{,}89$; $P = 0.29$ for males, high loading of CEHO). No significant differences were found between panel and ball traps (highest $F = 0.84$; $df = 1{,}64$; $P = 0.36$ for single degree-of-freedom contrast for males, low loading of CEHO). AMPu-baited traps were significantly more attractive than blanks in all four ANOVAs (lowest $F = 7.2$; $df = 1{,}64$; $P <$

0.01 for single degree-of-freedom contrast for males, low loading of CEHO). Neither loading of CEHO was significantly attractive to either males or females, although the numbers of flies captured by CEHO-baited traps were numerically higher than those captured by blank traps in each case (highest $F = 2.3$; $df = 1,64$; $P = 0.13$ for single degree-of-freedom contrast for females, low loading of CEHO). In the two ANOVAs for the high loading of CEHO, the AMPu × CEHO interaction was significant (lower $F = 4.0$; $df = 1,80$; $P < 0.05$ for single degree-of-freedom contrast for females). AMPu × CEHO interactions were not significant at the 5% level for either males or females for the low loading of CEHO, but the effect was similar to that of the high loading (lower $F = 1.9$; $df = 1,64$; $P = 0.17$ for single degree-of-freedom contrast for females). The interactions occurred because AMPu–CEHO combinations were less attractive than AMPu alone.

The statistical analyses presented above indicated no differences between ball traps and panel traps, or between low and high loadings of CEHO, with regard to captures of flies. Moreover, test day × lure interactions were not significant. Therefore, data were combined for ball and panel traps and for low and high loadings of CEHO. Test day × lure interactions were left out of the model. These simplified ANOVAs gave results that were essentially the same as in experiment 1. Traps baited with AMPu were significantly more attractive than blank traps or traps baited with CEHO to both male and female flies (Figure 2) ($F = 17.2, 41.2$; $df = 3,291$; $P < 0.0001$ for males and females, respectively). Traps baited with CEHO alone were not significantly more attractive than blank traps either by LSD or paired *t* tests. Traps baited with combinations of AMPu and CEHO were significantly less attractive than traps baited with AMPu alone to both male and female flies.

DISCUSSION

Combinations of AMPu with CEHO were either less attractive or no more attractive than AMPu alone in both experiments. This result cannot be attributed simply to the CEHO lures acting as repellents. Traps baited with membrane-based CEHO lures were not less attractive than blank traps, and PVC CEHO lures were more attractive than blank traps. Furthermore, in previous work, McPhail traps baited with membrane-based CEHO lures identical to those tested here were significantly more attractive than McPhail traps containing only water (Robacker and Heath, 1996).

Combinations of attractants that act on different appetitive behaviors, and perhaps on different central nervous system centers (Robacker, 1993), have usually proven less attractive than the more potent of the two attractants alone for *A. ludens*. The effect has now been demonstrated for combinations of pher-

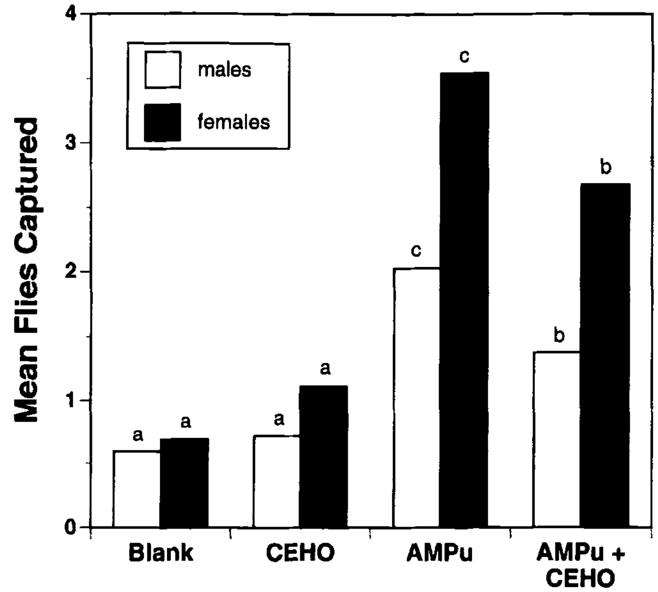

FIG. 2. Captures of *A. ludens* on sticky traps baited with a combination of an AMPu-agar lure and a membrane-based CEHO lure, compared with blank traps or traps baited with a CEHO or AMPu lure alone (experiment 2). Within each sex, bars with the same letter are not significantly different from each other by Fisher's protected LSD ($P < 0.05$; $N = 76$ for each trap/lure configuration).

omone with odor of fermented host fruit (Robacker and Garcia, 1990), chemicals from host fruit odor with bacterial odor (Robacker, 1991), and acetic acid with AMPu (Robacker et al., 1996). In each case, laboratory experiments indicated that the motivational state of the fly determined which of the two attractants of the combination elicited attraction and which inhibited the response to the combination. For example, sexually active female flies were strongly attracted to pheromone and less so to the combination of pheromone and fruit odor, whereas sexually immature, sugar-starved females were attracted more to fruit odor than to the combination (Robacker and Garcia, 1990). Similar decreased attraction to combinations of pheromone and protein baits was reported in *Bactrocera oleae* Gmelin (Haniotakis and Skyrianos, 1981; Haniotakis and Vassiliou-Waite, 1987; Zervas, 1989). However, several cases are known in Tephritidae in which combinations of attractants that apparently act on different appetitive behaviors were more attractive than either alone (Zervas, 1989; Landolt et al., 1992; MacCollom et al., 1992, 1994; Robacker et al., 1996). These findings indicate that attraction of fruit flies to semiochemicals is highly complex, involving interactions of chemicals with each other and various effects of physiological states of the flies on responses to the chemicals.

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